Characterization of the cell-uranyl complex of *Deinococcus radiodurans*

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Deinococcus radiodurans, the most extremely radioresistant bacterium known, is being studied for potential bioremediation of highly radioactive waste sites. Although the effects of UV and gamma radiation have been well studied, our cells will have to function well under mixed radiation fields for bioremediation. Previous studies establishing the radioresistance of *D. radiodurans* do not explicitly address the effects of many additional stressors likely to be encountered during bioremediation. We systematically studied the effect of growth phase, which is often key for controlled expression of engineered gene systems, on ^{60}Co γ radiation survivability.[1] We also studied the effects of radiation on sig factor knockout strains generated by collaborators and determined that at least one of the genes plays a role in survivability. In addition, with collaborators from the Life Sciences Division, we looked at the effects of high dose He and N radiation beams generated by the 88" cyclotron on cells in aqueous suspension and determined relative biological efficiency values of 1.5 and 2.5, respectively.[1] (Figure 1)

One potential system for *in situ* bioimmobilization or a waste stream bioreactor would be to precipitate dissolved actinides as insoluble phosphates on the cell surface. A gene system capable of building up phosphate and releasing it outside the cell has been constructed by collaborators, but unlike previous models using *Escherichia coli* and *Pseudomonas aeruginosa,*[2] no uranyl phosphate bioprecipitation was observed.[1] The difference in radiation survivability between these constructs and wild type cells was undetectable.

In order to better understand the cell-uranyl system, we studied the interaction of *D. radiodurans* with U(VI) in isotonic, pH 4.5 salt solution, conditions typical of bioreactors and *in situ* bioimmobilization. Kinetic studies indicate that under these conditions the cell loads uranyl to a concentration of about 20 mg U per g biomass (dry weight). The loading increases dramatically with pH as it rises above 4.0, (Figure 2) indicating deprotonation of sorbing groups at these pH values, which are typical of carboxylate rather than phosphate groups. The binding is reversible by acidifying the medium or washing with strong complexors such as citrate.[1] Previous characterization of the bacterial surface has shown that it has a highly crystalline surface layer (S-layer) of proteins with a high concentration of acidic residues displayed on the cell surface.[3] Laser fluorescence spectroscopy and infrared spectroscopy both seem to confirm the hypothesis of a carboxylate binding moiety complexing the uranyl.[2]

We wish to further characterize the cell surface system by repeating the FT-IR measurements and confirm that the surface carboxylates are indeed binding the uranyl using sum frequency IR techniques as well as selective covalent blocking of surface functional groups to identify which S-layer amino acid residues are involved in uranyl binding.

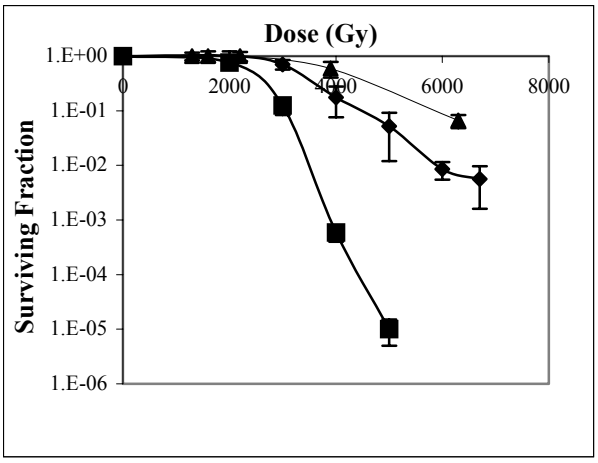

Figure 1: *D. radiodurans* survives particle radiation generated by the 88" cyclotron with a relative biological efficiency of 1.5 for He and 2.5 for N.

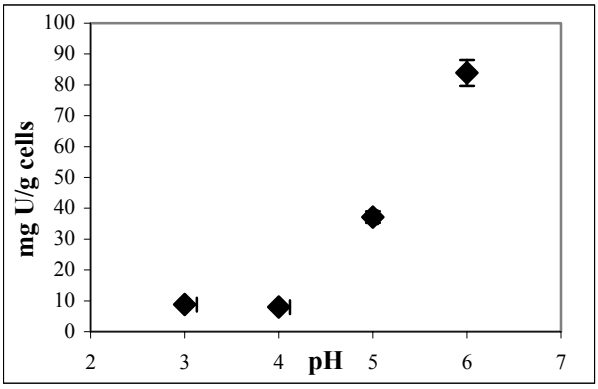

Figure 2: cell binding of uranyl rises dramatically with pH

REFERENCES

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